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Shapes of MHC Restriction

Review

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The defining component of antigen-specific recognition by T lymphocytes with αβ T cell receptors (TCRs) is MHC restriction. The concept of MHC restriction was derived from experiments which showed that virus-specific effector T cells could only lyse virus-infected target cells that were of the same MHC haplotype as the effector T cells (Zinkernagel and Doherty, 1974). Two models were proposed to explain the mechanism by which an uncharacterized TCR could be specific for both the foreign antigen (e.g., virus) and self-MHC molecules: (1) "altered self," in which a given MHC molecule was altered in an undefined, specific way that could be recognized by a single TCR; and (2) "dual recognition," in which two distinct receptors independently recognized the foreign antigen and the self-MHC molecule. Two subsequent discoveries favored the altered self hypothesis. First, MHC molecules were found to be peptide-binding molecules; each MHC molecule specifically binds a restricted set of peptides derived from foreign and self-proteins and presents them on cell surfaces to T cells (Townsend et al., 1985; Falk et al., 1991). Moreover, structural studies of peptide/MHC complexes showed that the surface of each complex was composed of MHC elements and amino acid side chains of the bound peptide that protruded upward out of the MHC molecule binding site where they could be contacted by a TCR (Fremont et al., 1992; Madden et al., 1993; Stern et al., 1994). Second, the TCR responsible for specificity for both the foreign antigen and MHC was shown to be a single cell surface heterodimeric protein composed of α and β chains (Hedrick et al., 1984; Yanagi et al., 1984; Yague et al., 1985; Dembic et al., 1986). The question posed by these discoveries was this: how does a single αβTCR embody specificity for both foreign (and self-) peptides plus the MHC? The most direct way to answer this question was to determine the protein structure of the TCR bound to a peptide/MHC complex.

The αβTCR is similar to an antibody Fab fragment in that it consists of two disulfide-linked chains, α and β, each containing a variable (V) and constant (C) domain. Each variable domain has three complementarity-determining regions (CDR1, CDR2, CDR3), with the highest sequence diversity occuring in the CDR3 region (reviewed in Davis et al., 1998). The first three-dimensional structures of a TCR β subunit and a Vα domain confirmed predictions that the TCR V and C domains are very similar to the corresponding structures observed in antibody molecules (Bentley and Mariuzza, 1996). Each of the three CDR loops protrude from the same face of the molecule. A fourth loop, termed HV4, is also present. The exception to these similarities has been the C domain of the α chain that has a noncanonical fold (Garcia et al., 1996; Ding et al., 1998). Structural studies of entire αβTCR molecules were slowed by the the inability to produce sufficient amounts of TCR protein to analyze, but in the last three years, several X-ray structures of human and murine αβTCRs complexed with peptides bound to MHC class I molecules have been determined. Two different human αβTCRs were identified in $CD8^+$ cytotoxic T cell clones (A6 and B7) specific for the Tax 11–19 peptide (LLFGYPVYV) of human T cell lymphotrophic virus type-1 (HTLV-I) presented by HLA-A2 and were isolated from the peripheral blood of HTLV-I-infected patients (Utz et al., 1996). The A6 and B7 α and β chains were individually expressed in bacteria, then refolded and bound to soluble forms of HLA-A2 complexed with the Tax peptide and crystallized (Garboczi et al., 1996a, 1996b; Ding et al., 1998).

A murine αβTCR was obtained from a $CD8^+$ cytotoxic T cell clone (2C) that was derived from alloimmunization of $H\text{-}2^b$ mice with $H\text{-}2^d$ cells and was initially shown to be specific for $H\text{-}2L^d$ (Kranz et al., 1984). The 2C TCR was subsequently shown to be able to recognize the self-peptide dEV8 (EQYKFYSV) derived from a mitochondrial protein presented by $H\text{-}2K^b$ (Tallquist et al., 1996). The α and β chains of 2C were expressed together in *Drosophila melanogaster* cells and were crystallized alone and complexed with soluble dEV8/$H\text{-}2K^b$ complexes (Garcia et al., 1996, 1998). Another murine αβTCR was obtained by a different group from a $CD8^+$ cytotoxic T cell clone (N15) that was derived from mice immunized with vesicular stomatitis virus (VSV) and shown to recognize the VSV8 peptide (RGYVYQGL) presented by $H\text{-}2K^b$ (Shibata et al., 1992). The α and β chains of N15 were expressed together in Chinese hamster ovary cells (Liu et al., 1996) and crystallized with and without the soluble VSV8/$H\text{-}2K^b$ complexes. Although the N15 TCR structure is determined to 2.8 Å resolution (Wang et al., 1998), the report of the N15/$H\text{-}2K^b$ structure is of low resolution (Teng et al., 1998).

Orientation of αβTCRs on the Peptide/MHC Complex

Each of the Tax peptide–specific TCRs (A6 and B7) and the 2C TCR bind to the peptide/MHC complexes in a diagonal orientation. A similar mode of binding was observed in the N15 TCR/$H\text{-}2K^b$ complex. The TCRs bind diagonally between the high points on the α1α2 helices of the MHC molecule (Figure 1). It appears that this alignment of TCR and MHC is necessary in order for the TCR to interact with the relatively small area of peptide surface that is not buried in the MHC molecule (Garboczi et al., 1996b; Ding et al., 1998). Bound to the MHC molecule in this way, the CDR1 loop of the α chain is positioned over the N-terminal end of the peptide, and the

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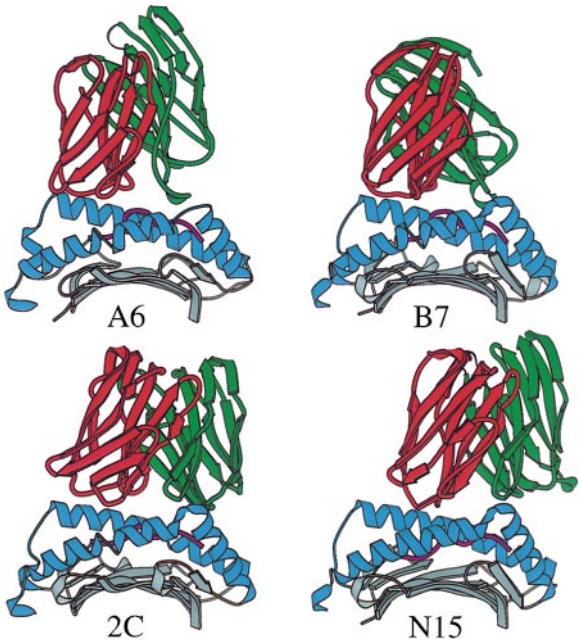

Figure 1. Side View of the Four Known TCR/MHC Orientations

Ribbon depiction of the human A6 and B7 and the mouse 2C and N15 TCRVαVβ regions oriented on their respective MHC complexes. The VαVβ domains are shown in red (Vα) and in green (Vβ). In blue are the helices (front, α2; back, α1) of the MHC molecules. In gray is the β sheet forming the floor of the MHC peptide-binding site. This figure was made by superimposing (program LSQMAN [Kleywegt, 1996]) each MHC α1α2 domain on to the α1α2 domain of HLA-A2 using coordinates deposited at the Protein Data Bank (PDB) (accession numbers 1ao7, 1bd2, and 2ckb) and from J.-H. Wang (Teng et al., 1998) and by depicting the orientations with the program MOLSCRIPT (Kraulis, 1991). Not shown are the constant domains of the TCR and the α3 and β_2m domains of the MHC molecule.

CDR1 loop of the β chain is over the C-terminal end of the peptide. The CDR2 loop of the α chain is positioned over the MHC α2 helix, and the CDR2 of the β chain lies over the MHC α1 helix. The CDR3 loops of both α and β chains combine to form a central pocket that can bind a peptide residue that extends from the surface of the MHC molecule. This diagonal orientation has two important consequences for TCR recognition. First, it places the part of the TCR with the most extensive sequence diversity (CDR3s) directly over the center of the MHC/peptide complex, which permits direct binding to any central peptide side chain that protrudes out of the MHC binding site. This feature of TCR recognition suggests that peptide side chains that are protruding from the middle of the MHC groove will be most important for TCR recognition. Second, it places the germline-encoded CDR2 loops over each of the MHC α helices, which provides a docking mechanism that will not be disrupted by the somatic diversification mechanisms that contribute to CDR3 diversity. In each of the three TCR/peptide/MHC complexes (A6, B7, and 2C), multiple atomic contacts exist between amino acid residues in the CDR2α or CDR2β loops and conserved amino acid residues on the α1 and α2 helices of the MHC. Since the CDR2α and CDR2β segments from different Vα and Vβ genes have conserved amino acid motifs (Arden, 1998), it is possible that conserved CDR2 motifs provide a commonality which permits different V region CDR2s to bind to any class I molecule that displays its own conserved residues on the α1 and α2 helices. Such a conserved docking structure could provide an explanation for the difficulty in inducing MHC-restricted antibodies. While antibody CDR3 loops could bind to the peptide side chains that point up out of the MHC molecule, antibodies do not have the conserved sequence motifs of the CDR2 loops of TCR V segments and may thus have difficulty docking onto the MHC molecule.

To date, a structure has not been reported of the complex of a TCR and a peptide/MHC class II molecule, so it is not yet known if this diagonal mode of TCR interaction with peptide/MHC class I molecules will be applicable to class II interactions. However, studies on class II–restricted TCR recognition using TCR mutagenesis (Engel and Hedrick, 1988; Katayama et al., 1995) and TCR selection by variant peptide immunization (Jorgensen et al., 1992; Sant'Angelo et al., 1996) all provide indirect evidence that the use of such a diagonal binding mode will also be true for class II–restricted TCR recognition.

TCR Interactions with Peptides

In theory, the TCR could interact with any part of an MHC-bound peptide that is not buried in the MHC molecule; moreover, specificity of the TCR for total peptide binding could be a sum of specific interactions with many peptide side chains. However, this hypothesis was not borne out by the data (see Table 1). Most of the TCR contacts with peptide involve only one or two peptide

Table 1. Contacts between Residues of the Tax and dEV8 Peptides and Their TCRs

Tax Peptide	P1 L	P2 L	P3 F	P4 G	P5 Y	P6 P	P7 V	P8 Y	P9 V	Total
A6 TCR	1	1	0	9	18	3	5	12	0	49
B7 TCR	2	0	0	6	26	1	8	13	0	56
dEV8 Peptide		P1 E	P2 Q	P3 Y	P4 K	P5 F	P6 Y	P7 S	P8 V	Total
2C TCR		0	0	0	19	0	5	4	0	28

Atoms of peptide and TCR amino acid residues that are less than 4 Å apart were listed by the program CONTACT (CCP4, 1994) using TCR/peptide/MHC coordinate files (legend to Figure 1) as input and tabulated here. For example, for the P5 tyrosine of the Tax peptide there are 18 atom–atom pairs in which an atom of the tyrosine residue (main chain or side chain) and an atom of the A6 TCR are positioned less than 4 Å apart.

amino acids. The A6 and B7 TCRs contact similar amino acid side chains of the Tax peptide (Table 1, see legend for definition of contact). Both receptors have the majority of their contacts with the tyrosine residue at position 5 (P5) of the peptide, binding the side chain in the central cavity formed by the CDR3α and CDR3β loops (see Figure 3, below). Both Tax peptide–specific TCRs focus almost exclusively on two amino acid side chains (P5Y and P8Y) that protrude from the HLA-A2 peptide binding groove. The conformation of the bound Tax peptide is essentially the same in both the A6 and B7 TCR/peptide/MHC complexes. Comparison of the structure of the Tax peptide as determined in the complex with soluble HLA-A2 without the TCR (Madden et al., 1993) shows that both the A6 and B7 TCRs substantially deform the P6 and P7 residues of the peptide (Garboczi et al., 1996b; Ding et al., 1998).

In contrast, the 2C TCR forms many fewer atomic contacts with the dEV8 peptide (Table 1). The large central cavity of the 2C TCR formed by the CDR3α and CDR3β loops is not occupied by a peptide side chain. The majority of the atomic contacts are with the upward-pointing P4 Lys. In addition, the interface of the 2C TCR with the dEV8 peptide shows a rather low degree of complementarity and quite distal peptide contacts (Garcia et al., 1998). Together, these properties of the 2C TCR dEV8/K^b interface may explain the relatively weak affinity of this particular interaction ($K_D \sim 8 \times 10^{-5}$ M [Garcia et al., 1997]) as well as the ability of self-K^b molecules to positively select the 2C TCR in TCR transgenic mice (Sha et al., 1988). If the 2C TCR/dEV8/H-$2K^b$ interaction is typical of TCR/selecting self-peptide interactions in general, then positive selection will occur only if the TCR has a similar "poor fit" to the endogenous peptide/MHC complex that it encounters during thymic selection. A "better fit" interaction, as typified by A6 or B7 TCRs with the Tax/HLA-A2 complex, could then lead to negative selection.

The observation that all three TCRs focus on only a few peptide amino acids also helps to explain the phenomenon of altered peptide ligands (APLs). APLs were originally defined as agonist peptides with single amino acid substitutions that altered T cell responsiveness (reviewed in Sloan-Lancaster and Allen, 1996). Certain amino acid substitutions produced partial agonists that were able to stimulate some, but not all, of the T cell responses induced by the agonist ligand. Other amino acid substitutions produced antagonists that could induce downregulation of responses to the agonist peptide. The partial agonist and antagonist patterns of T cell responsiveness were accompanied by differential signal transduction as revealed by the phosphorylation patterns of the TCR-associated ζ chains and ZAP-70 (Sloan-Lancaster et al., 1994; Madrenas et al., 1995). Partial agonist and antagonist peptide/MHC complexes were shown to bind to TCRs with a lower affinity than the agonist complexes (reviewed in Davis et al., 1998). The structural data from the three TCR/peptide/MHC complexes described above suggests that this APL effect is a consequence of peptide substitutions that disrupt some of the contacts with the TCR and reduce binding to the one or two amino acids that comprise the most contacts with the peptide. For example, substitution of the P5 Tyr of Tax with Ala induces a partial agonist response for clone A6 (Garboczi et al., 1996a). It can be seen (Table 1) that such a substitution changed the amino acid that made the most contacts to the Tax peptide by the A6 TCR.

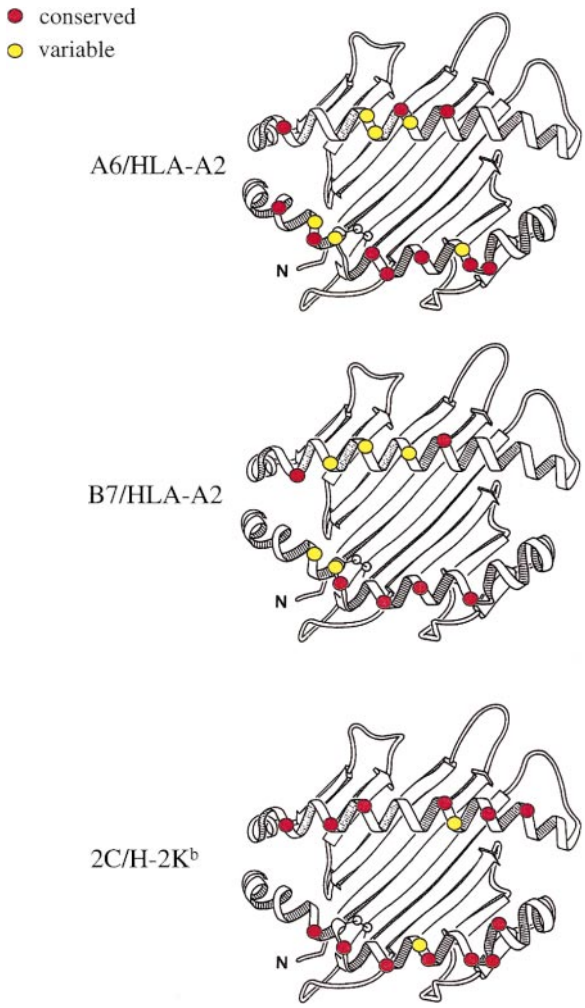

Figure 2. Conserved and Variable MHC Residues Contacted by TCRs

Ribbon diagrams of class I molecules show the location of MHC amino acids that are contacted (within 4 Å) by the A6, B7, or 2C TCRs and are either conserved (red circles) or are variable (yellow circles) in HLA-A alleles or in H-2K alleles (also see Table 2). The α1 helix is at the top and the α2 helix is at the bottom. N, amino terminus of MHC heavy chain. The disulfide bond between residues 101 and 164 is shown.

TCR Interactions with MHC Molecules

One question that arises from these studies is this: since the TCR focuses on just a few of the peptide amino acids, is it also true that the TCR focuses on only a few amino acids on the MHC molecule? The answer appears to be no, since each of the three TCRs has multiple contacts with MHC amino acid residues on the α1 and α2 helices (Figure 2). The majority of the contacts are with conserved amino acids in each MHC allele (Figure 2; Table 2). For example, the 2C TCR contacts two variable amino acids but binds to 13 conserved residues on the K^b molecule (Figure 2). Thus, for each of the three TCR/

Table 2. Contacts between MHC Residues and TCR α and β Subunits

HLA-A	HLA-B	HLA-C	HLA-A2 Residue	A6 α M	A6 α S	A6 β M	A6 β S	B7 α M	B7 α S	B7 β M	B7 β S
E	E	E	58E	0	9						
Y	YH	Y	59Y					1	0		
GRQEL	GR	R	62G					1	0		
RG	RQ	Q	65R	0	13			0	14	0	1
KN	INK	KN	66K	0	6						
K	K	KN	68K	1	0						
A	TAR	R	69A	1	3			0	1		
Q	Q	Q	72Q			0	1			0	1
AT	A	A	149A			2	0				
AV	A	A	150A			14	3			1	1
HR	R	R	151H			0	1				
Q	Q	QE	155Q	0	1	0	5	0	1	0	1
AV	AT	A	158A	0	1			7	4		
Y	Y	Y	159Y	0	1						
G	GD	G	162G					2	0		
TREL	TLE	TLE	163T	0	2			0	1		
ED	ED	E	166E	0	7						
WG	WGS	W	167W	0	3			0	3		
R	R	RG	170R	0	4						
				2	50	16	10	11	24	1	4
				52		26		35		5	

HLA-A2 residues (i.e., 58E, 59Y, 62G, . . .) are those contacted (<4 Å) by either the A6 or B7 TCRs. Residues found in other HLA-A, -B, and -C alleles are shown (Kostyu et al., 1997). Columns headed by "M" or "S" list the number of contacts from a TCR subunit to main chain or side chain MHC atoms, respectively. Contacts were identified as described in the legend to Table 1.

MHC interfaces described thus far, the predominant interactions are with multiple conserved amino acids on the α1 and α2 helices of the MHC. These findings suggest that conserved MHC residues play a critical role in TCR interactions and implies that TCRs were evolutionarily selected for their ability to recognize conserved features of MHC molecules (Janeway et al., 1997; Zerrahn et al., 1997). Knowing the relative energetic contributions of each contact will require mutagenesis and quantitative binding studies.

The TCR α chain mediates most of the contacts with the MHC molecule (Table 2, for 2C, data not shown). The A6 and B7 α subunits show more interactions with the side chain atoms of HLA-A2 than with main chain atoms, especially with the Arg side chain at position 65 (65R) of the α1 helix (Table 2). In the A6 β chain, contacts are about evenly divided between MHC main and side chain atoms, with a principal focus on the main chain atoms of the Ala at position 150 (150A). In contrast, the B7 β chain makes very few contacts with the MHC molecule. Thus, these two different TCRs recognize the same MHC molecule through structurally distinct interactions. These findings imply that multiple solutions exist for the problem of MHC-restricted TCR recognition of a particular MHC molecule and also indicate the presence of extensive diversity in the capacity of TCRs to effectively bind MHC molecules once they have achieved the diagonal binding orientation.

Now that we have observed the diverse mechanisms by which these TCRs recognize their respective peptides and MHC molecules, the answer to the original question seems clear: the TCR makes separate contacts with distinct components of the peptide and of the MHC molecule. This solution is somewhat like the original dual recognition model except that a single receptor is utilized to separately recognize foreign and self-antigens. These findings refute one of the extreme versions of the altered self model, which postulated that no recognition of a foreign component by TCRs occurred, but only a conformationally altered self-MHC molecule that resulted from binding of the foreign element.

Comparison of A6 and B7 TCR/Tax Peptide/HLA-A2 Complexes

Just as the A6 and B7 Tax-specific T cells recognize the same MHC molecule through structurally distinct interactions, these two T cells also recognize the Tax peptide differently. This conclusion was derived from studies which showed that A6 and B7 differentially recognized Tax peptides with single amino acid substitutions at TCR contact residues (Ding et al., 1998). By comparing how these TCRs bind the same peptide/MHC, we could address the question of how two different T cell receptors recognize the same peptide/MHC in different ways. The most remarkable feature revealed by a comparison of these two structures is that only one of the 17 TCR amino acids in B7 that contact the peptide/MHC complex is shared with the A6 TCR (CDR2α51S). This amino acid diversity produces significantly different molecular contact surfaces on the A6 versus B7 TCRs (Figure 3). Most notable is the central cavity of each TCR, which binds the P5 Tyr of the Tax peptide (Figure 3). This cavity is positively charged in A6 (blue in Figure 3) and negatively charged and smaller in B7 (red in Figure 3). These differences in the molecular contact surfaces of the central cavity explain why amino acid substitutions at P5 in the Tax peptide differentially affect recognition by the A6 and B7 TCRs (Ding et al.,

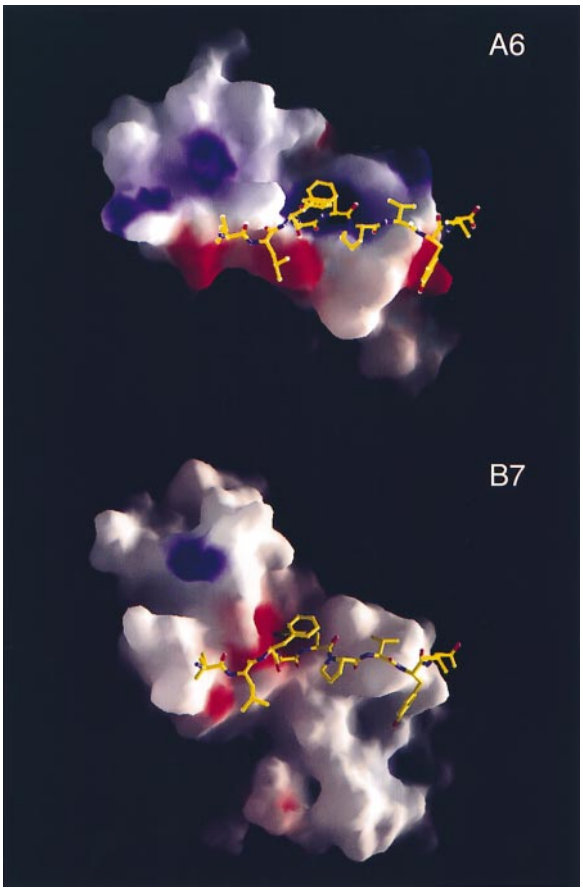

Figure 3. Comparison of A6 and B7 TCR Surfaces that Contact the Tax Peptide

The Tax peptide is shown as a ball and stick model, and the electrostatic charge potential of the TCR surfaces is shown in red for negative, blue for positive, and white for neutral. The figure is drawn with the program GRASP (Nicholls et al., 1991).

1998). The direct demonstration that two TCRs with very different contact surfaces can bind the same peptide/MHC complex indicates that a diverse repertoire of TCRs can recognize any single peptide/MHC complex. This feature of the TCR provides an explanation for the diverse array of αβTCRs observed in Tax peptide/HLA-A2-specific T cell clones derived from three HTLV-1-infected patients (Utz et al., 1996). In addition, flexibility in the chemistry of the contact surface of the TCR also provides an advantage for the host immune response to small protein antigens. Such small proteins will generate relatively few peptides, only a small fraction of which will be of the appropriate length and sequence capable of binding a particular MHC molecule with sufficient affinity to produce enough complexes on the cell surface to trigger TCRs. If only a very limited number of TCRs that could bind a particular peptide/MHC complex were available, then it is likely that there would be no response to such small protein antigens. The property of having a structurally diverse set of TCRs that can recognize the same peptide/MHC complex in different ways expands the available repertoire for recognition of any protein.

Structural Flexibility of the TCR

Do the CDR loops of the TCR have the ability to move to accommodate binding to peptide and MHC components? The structure of the 2C TCR was initially determined on an unliganded form of the receptor (Garcia et al., 1996) and was determined again when bound to the dEV8/K^b complex (Garcia et al., 1998). Comparison of these two structures yielded important findings about the structural consequences of ligand binding and the flexibility of the contact surfaces (Garcia et al., 1998). Although no major domain rearrangements were found in the liganded form, large conformational adjustments were observed in the CDRs that contacted the peptide. This structural flexibility of the CDRs that contact peptide could explain the capacity of a single TCR to accommodate structurally diverse peptides and thereby produce the phenomenon known as cross-reactivity or molecular mimicry. In contrast, CDRs that were primarily involved in contact with the MHC α helices did not move significantly. This apparent lack of movement in the CDRs that interact with the MHC indicates their involvement in conserved interactions (Garcia et al., 1998). This issue is particularly pertinent to the observation that most MHC residues that are contacted by all three TCRs are conserved (Figure 2) and provides a structural explanation for the finding that the predominant TCR/MHC interactions are with conserved elements on MHC molecules.

Recent studies on TCR recognition have begun to emphasize the importance of a significant level of cross-reactivity on different peptides, which is necessary to produce an adequate TCR repertoire capable of responding to the universe of pathogens (reviewed in Mason, 1998). Development of the TCR repertoire during thymic selection occurs on a set of self-peptides bound by self-MHC molecules, but these self sequences may have little or no sequence homology with pathogen-derived peptides. Also, activation of autoreactive TCRs can occur by recognition of pathogen-derived peptides that possess little or no primary sequence homology with the selecting self-peptide (Wucherpfennig and Strominger, 1995; Hemmer et al., 1997). The TCR/peptide/MHC structures determined to date indicate that the required degree of peptide cross-reactivity can be produced in at least two ways: (1) the TCRs focus on only a few amino acid side chains of the peptide and can accommodate peptides with other side chains, depending on the size and surface chemistry of the TCR contact surfaces; and (2) the structural flexibility of the CDR3 loops that contact the peptide allow a degree of accommodation of binding to multiple peptide ligands. Future studies of the structures of the same TCR bound to structurally diverse peptides presented by the same MHC molecule should provide further insights into the biophysical basis of TCR cross-reactivity.

Structural Implications for TCR Signaling

The purpose of TCR ligand binding is to initiate signal transduction that results in the activation of functional programs within the cell. How does binding of ligand on the extracellular V domains of the TCR translate into a functional message inside the cell? One model to explain

these events is that ligand binding by the TCR V domains induces a conformational change in the C domains that, in turn, changes the dynamics of the interactions with CD3 chains and/or ζ chains associated with the receptor and thus transduces a signal through these associated components. However, a comparison of the liganded and unliganded forms of 2C showed that the conformational changes that occurred in the TCR as a result of antigen binding did not appear to involve the constant domains of the molecule (Garcia et al., 1998). In addition, comparison of the Cα-Cβ interfaces of the liganded B7 TCR with unliganded 2C and N15 showed that they were nearly identical (Ding et al., 1998). Thus, the current structural evidence does not support the conformational change model.

An alternate model for signal transduction by the TCR postulates that ligand binding induces receptor aggregation and/or oligomerization that segregates the bound receptors into cell–cell attachment patches (Davis et al., 1998). Such receptor aggregation would facilitate clustering of intracellular components required for the recruitment and phosphorylation of signal transduction components. The adoption of a uniform orientation in TCR binding to peptide/MHC complexes may provide a mechanism that facilitates TCR aggregation and interactions with the coreceptors (CD3s, CD4/CD8) that can generate a T cell signal (Davis et al., 1998). At this juncture, no evidence for dimerization or oligomerization of liganded TCRs is provided from the crystallographic data. However, the conditions used to produce these complexes may not favor multimerization, whereas the conditions of cell surface TCRs associated with CD3s/ζ chains could be much more favorable. Future structural studies of unliganded and liganded TCRs in association with the CD3 chains and ζ chains could provide new insights into the mechanisms of the initiation of signal transduction by the TCR.

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